Original Article

Expression of Heat Shock Protein (HSP A1A) and MnSOD Genes Following Vitrification of Mouse MII Oocytes with Cryotop Method

Zahra Khodabandeh Jahromi, M.Sc.[1], Fardin Amidi, Ph.D.[1]*, Seid Mohammad Hossein Nori Mugehe, Ph.D.[1], Aligoli Sobhani, Ph.D.[1], Kobra Mehrannia M.Sc.[1], Mehdi Abbasi, Ph.D.[1], Mehryar Habibi Roudkenar, Ph.D.[2], Afruz Habibi Ph.D.[3], Majid Ebrahimi, M.Sc.[2]

1. Anatomy Department, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Research Center, Iranian Blood Transfusion Organization, Tehran, Iran
3. Anatomy Department, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

** Corresponding Address: P.O.Box: 1417613151 , Anatomy Department, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
Emails: famidi@sina.tums.ac.ir

Received: 19/Nov/2009, Accepted: 9/Feb/2010

Abstract

Objective: The aim of this study was to investigate the effects of two vitrification protocols on mouse metaphase stage II (MII) oocytes and evaluate their effects on the expression of heat shock protein A1A (HSP A1A) and MnSOD genes.

Materials and Methods: Groups of approximately 15 oocytes without cumulus complexes were collected and vitrified with 10% (v/v) ethylene glycol (EG) + 10% (v/v) dimethylsulphoxide (DMSO) + 0.5M sucrose in group A (VSI) and 14.5% (v/v) EG + 14.5% PROH + 0.5M sucrose in group B (VSII), respectively. Thawing after vitrification was performd by placing the oocytes into 1M sucrose for 1 minute and two diluted solutions, each for 3 minutes. After thawing, the oocytes were fertilized and cultured *in vitro* to develop into the pronuclear stage. The survival rate of vitrified-warmed oocytes and rate of fertilization were evaluated. In addition, gene expressions (HSP A1A, MnSOD and β actin) of vitrified-warmed oocytes were also examined by reverse transcription polymerase chain reaction (RT-PCR).

Results: Survival rates of each group were separately compared to the control. The result showed significant differences between each experimental group compared to the control ($p \leq 0.001$). The survival rate of oocytes after warming was higher in VSI (91.2% ± 1.7) compared to VSII (89.2% ± 1.5) but there were no significant differences between the two groups. The rate of fertilization was significantly ($p \leq 0.05$) reduced in vitrified-warmed (VSI: 39% ± 5.8; VSII: 34% ± 5.7) oocytes compared to the control (88.36% ± 2.3). The expression of MnSOD increased in the vitrified-warmed oocytes when compared to control oocytes. We also detected HSP A1A only in the control and VSI group.

Conclusion: Vitrification of oocytes by cryotop resulted in high survival rate; low developmental competence and fertilization rate of vitrified-warmed oocytes which may be a result of changing expression of important genes after thawing.

Keywords: Oocyte, Vitrification, Cryotop, Gene Expression, HSP A1A, MnSOD

Yakhteh Medical Journal, Vol 12, No 1, Spring 2010, Pages: 113-119

Introduction

Oocyte cryopreservation as an essential part of assisted reproductive technology (ART) has recently received major success in clinical practice (1, 2). This technology is used to preserve fertility in young women that are at risk of losing ovarian function for a variety of reasons such as chemotherapy or radiotherapy for cancer and autoimmune or hematologic diseases (3, 4).

Although sperm and embryo freezing has greatly improved, oocyte cryopreservation still faces numerous problems (5). At present, vitrification is a feasible method for cryopreservation of oocytes or embryos (2). In this procedure, glasslike solidification is achieved by using a high concentration of cryoprotectants (CAPs) and a rapid cooling rate (6). For the first time, Rall and Fahy used vitrification to freeze mice embryos (7). Several vitrification protocol were proved according to different types of tools, including the open pulled straw (8), electron microscope grid (9), cryoloop (10), and cryotop (11) in addition to different cryoprotectant solutions (6, 12).

Among the various cryodevices, the cryotop as devised by Kuwayama has recently attracted more attention since a minimum volume of solution (ap-

proximately 1µl) is loaded onto it, thus achieving both a high cooling (-23000˚C/min) and warming (40000˚C/min) rate(15). Furthermore the concentration of permeable CPAs is decreased to 30% by this method, thus minimizing the potential toxic effect (13-15). Based on further studies the cryotop is an easy and reliable procedure (15). Lucena et al.(16) achieved an 89% survival rate and up to 56% pregnancy rates with this method.

The most common CAPs used for the vitrification method are ethylene glycol (EG), dimethylsulphoxide (DMSO) and 1,2-propanediol (PROH) as permeable and sucrose as nonpermeable CAPs (17, 18). Kuwayama et al. have reported that the combination of DMSO and EG in equal proportions was the most efficient (15). Furthermore, the low molecular weight, high permeation ability and low toxicity of EG have enabled it to be used for vitrification of human oocytes and embryos. DMSO can facilitate EG permeability, cause increased polyploidy and may cause spindle polymerization whereas PROH is more permeable compared to DMSO (19). In addition, Vajta et al. have reported that cryoprotectant mixtures when compared to solutions that contain only one permeable cryoprotectant can decrease individual specific toxicity (8, 20). Moreover, osmotic stress may be induced by the use of a high concentration of CPAs (21), thus, the combination of a low concentration of CAPs can reduce this negative effect.

During the past decade, further studies have reported only structural and morphological damages such as zona hardening, modification in selective permeability of the plasma membrane, aneuploidy and nuclear fragmentation in the vitrified-warmed oocytes during vitrification (5).

Accordingly, there is little information on biological and molecular events following cryopreservation of oocytes (5, 22). Heat shock protein (HSP A1A(has a protective function by heat or stress response on the cellular auto regulation. The critical role of HSP A1A as a protective protein due to external stress has been proven. It was demonstrated that knock out HSP 70.1 mice had higher sensitivity to osmotic stress after preconditioning with heat was decreased (23-25). Hut et al. observed that HSP A1A has a protective role in the mitotic cell and prevented chromosomal division that occurs following heat-induced centrosome damage (26). Manganese super oxide dismutase (MnSOD) is an anti-oxidant enzyme that protects oocytes and embryos against oxidative stress damage. It has been shown that addition of antioxidant enzymes such as catalase or SOD1 (Cu-Zn-SOD) to culture media lead to an increased rate of blastocyst formation in the rabbit (27), mouse (28), and bovine species (29).

Sona et al. have reported that cold stress can be influenced on expression of several genes that are related to stress (30). Succu et al. (31) found that vitrified oocytes have shown low developmental competence after *in vitro* fertilization (IVF) and *in vitro* culture (IVC) Thereby for evaluating the post warming quality of oocytes, this is the best way to focus on alternation of gene expression related to stress and important cell function in response to cooling.

To the best of our knowledge, gene expressions have not been completely investigated in mammalian vitrified oocytes. The objective of present study was to determine expression of some genes related to stress including HSP A1A and antioxidant enzyme MnSOD. On the other hand, the efficiency of the cryotop method with the mixture of CAPs on survival, morphology, IVF and gene expression of mouse oocytes were examined.

Materials and Methods

All chemicals and media were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA), unless otherwise indicated.

Oocyte collection

In this study, 8-10 week old female mice (NMRI strain) were used. The mice were kept under the a 12 hours light: 12 hours dark cycle for two weeks to adapt to laboratory conditions. The animals were superovulated by intraperitoneal injections of 10 IU PMSG and followed 48 later by 10 IU HCG. The mice were sacrificed by cervical dislocation 13-15 hours post-HCG administration.

The oocyte cumulus complexes were taken from the ampulla of oviduct and put in hepes-buffered M199 medium + 20% fetal bovine serum (FBS) (Gibco). Denudation of oocytes were done by placing the oocyte cumulus complexes in M199 medium containing 300 µg/ml hyaluronidase for 30 seconds to 1 minute and by repeated pipetting in the holding medium (M199 + 20% FBS). Normal MII stage oocytes were indicated by the presence of a first polar body under the light microscope.

Vitrification

M199 supplemented with 20% FBS was used as a carrier for the cryoprotctants. Denuded MII oocytes were vitrified in groups of 15 by the minimum volume cooling (MVC) method using the cryotop as a device (14). The complete vitrification process was accomplished at room temperature (25°C). In the present study, a mixture of two concentrations

of vitrification solution (VS) were used. In group A, VS1 supplemented with 10% ethylene glycol (EG) + 10% dimethylsulphoxide (DMSO) + 0.5 M sucrose in base medium. In group B, VS2 consisted of 14.5% EG + 14.5% PROH in base medium. The equilibration solutions were 50% VS1 and VS2 which contained no sucrose.
Oocytes were primarily exposed to first equilibration drop for 3 minutes, after which they were merged with an adjacent ES drop. Subsequently, the oocytes were transferred to the vitrification solution for 1 minute. Then, oocytes were quickly loaded onto the top of the cryotop (Kitzato Ltd. Tokyo, Japan). All excess media were carefully removed and the cryotops were promptly submerged in liquid nitrogen (LN2).

Thawing
Upon warming, the cryotops were instantly inserted into the thawing solution (1M sucrose, M199 + 20% FBS) for 1 minute at 37°C. Subsequently, oocytes were placed into decreasing sucrose concentrations (0.5 M, 0.25 M) for 3 minutes each, then twice washed in holding medium for 5 minutes for cryoprotectant removal. The survival rate of vitrified-warmed oocytes was assessed on the basis of normal appearing zona pellucida, an integral plasma membrane and intact polar body as visualized under light microscope.

IVF
Survived epididymal vitrified, warmed oocytes were chosen and placed into 200 µl drops of IVF medium (HTF + 15 mg/ml BSA) layered with mineral oil. The medium was prepared previously and equilibrated at 37°C in a 5% CO_2 incubator.
Suspensions Ham's F-10 of epidydimal sperm were incubated for 45-60 minutes in capacitation medium (ham's F10 + 4 mg/ml BSA). A final concentration of 2×10^6 spermatozoa/ml were added to IVF medium which contained 10-15 oocytes and incubated at 37°C in 5% CO_2.
After 26 and 32 hours post-fertilization, the number of cleaved oocytes that developed into the pronuclear stage was recorded.

RNA extraction
Total RNA was extracted from 20 vitrified and non-vitrified oocytes with tripure isolational reagent (Roche, Germany) according the manufacturer's instructions. The concentration of extracted RNA and purity were determined by ND-1000 spectrophotometer (Nonodrop, Wilmington, DE). RNA was subsequently subjected to reverse transcription polymerase chain reaction (RT-PCR) which was performed using cycle script RT premix (dN_6) reverse transcriptase (Bioneer, South Korea) with 300ng of total RNA following the manufacturer's protocols.

PCR
The cDNA equivalents of 20 oocytes were used in each reaction. PCR was performed using Taq polymerase enzyme (Roche, Germany).
PCR analysis was carried out in a total volume of 25µl that consist of 1µl of each primer mix, 2µl dNTP, 2.5µl 10x buffer with $MgCl_2$, 0.3µl rTaq polymerase enzyme, 1µl cDNA and 18.2µl DDW. After an initial denaturation step of 3 minutes at 94°C, amplification of 20 oocytes was performed. Denaturation in each cycle included: 94°C for 30 seconds and annealing at 55 °C for MnSOD, and 59°C for HSP A1A for 30 seconds and extension at 72°C for 1 minute.
PCR primers were designed using primer 3 software based on mouse DNA sequences found in the Gene Bank (NCBI). Primers were subsequently put into BLAST search to examine the aligned seqences for polymorphisms and to avoid these regions for primers or probe design (Table 1).
RT-PCR products underwent electrophoresis on a 2% agarose gel, then were stained by ethidium bromide (Cina gene, Iran) and visualized under ultraviolet.

Statistical analysis
Oocyte survival and fertilization rates were analysed by ANOVA. Vitrification and *in vitro* fertilization data were analyzed by SPSS version 11.5 software.

Results

Vitrification and IVF
The results are summarized in table 2. After thawing, the survival rate of oocytes was high and there was no significant difference between VSI (91.2% ± 1.7) and VSII (89.2% ± 1.5). Survival rate of each group was seperately compared to the control. The results showed significant differences between each experimental group compared to the control ($p \leq 0.001$). The survival rate of oocytes after warming was higher in VSI (91.2% ± 1.7) compared to VSII (89.2% ± 1.5) but there were no significant differences between the two groups. The survival rates of oocytes were higher in VSI (91.2% ± 1.7).
As shown in table 2, the *in vitro* fertilization rates were significantly decreased in vitrified-warmed oocytes (VSI: 39% ± 5.8; VSII: 34% ± 5.7) compared to the control (88% ± 2.3). Although the fertilization rates were not different between the

Table 1: Information on the primers used for PCR amplification

Size (bp)	Location	Annealing temperature (ºC)	Sequence	Gene bank accession number	Gene
190	650-839	60	5'tcatgaagatcctcaccgag3' 5'ttgccaatggtgatgacctg3'	NM_001101 NM_001101	B actin
161	237-398	55	5'ggaagccatcaaacgtgact3' 5'ccttgcagtggatcctgatt3'	NM_001024466.1 NM_001024466.1	SOD_2
194	668-862	59	5'cgacctgaacaagagcatcaac3' 5' tgaagatctgcgtctgcttggt3'	ENST00000375651	HSPA1A

Table 2: Effect of the cryotop method by using two various vitrification solutions on survival and fertilization rate of MII oocytes (Mean ± SEM)

Treatment	No. of oocytes	% Survived oocytes	% Fertilization rate
Control	136	100 ± 0.0^{a}	88.0 ± 2.3^{a}(131)
VSI(EG + DMSO)	135	91.2 ± 6.7^{b}	39.0 ± 5.8^{c}(58)
VSII(EG + PROH)	133	89.2 ± 6.1^{b}	34.0 ± 5.7^{c}(48)

^{abc} ***Different superscripts indicate statistical difference (ANOVA: a vs. b = p≤ 0.001, a vs. c = p ≤ 0.05).***

two experimental groups, the results showed significantly lower fertilization rates compared to the control (p≤0.05). The fertilization rate was higher in VSI (39% ± 5.8) compared to the VSII (34% ± 5.7) group.

Gene expression analysis

As shown in figure 1 the expressions of all genes in the vitrified-warmed oocytes were compared to the control. RT-PCR was prepared to investigate the alternation in gene expressions. The abundance of mRNA was generally reduced in oocytes related to vitrification procedures, but expression of MnSOD increased in vitrified-warmed oocytes when compared to control oocytes. HSP A1A was only detected in the control and VSI group.

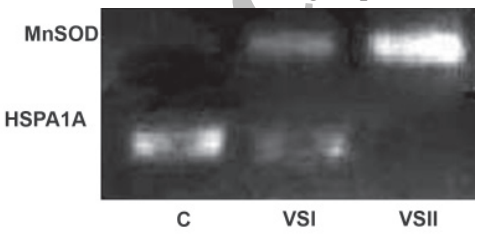

Fig 1: Gene expression of MnSOD and HSP A1A were measured by RT-PCR.

Discussion

In the present study we observed that the expression of a novel member of HSP70 family, HSP1A1, and an antioxidant enzyme, MnSOD, were altered in vitrified-warmed MII oocytes when compared to the control. A decrease in fertilization rate of the vitrified-warmed group after IVF was also seen. Intrinsic factors in oocytes are responsible for controlling the rate and time of cleavage. According to studies that have been performed in the animal model, decreasing fertilization rate and low developmental competence of oocytes after warming may be associated with alternations in expressions of antioxidant enzymes (32, 33) and cryoprotectant toxicity (34, 35). The developmental competence of oocytes depend on the abundance of specific gene transcripts (36-38).

The genes we chose are involved in response to stress (MnSOD, HSP A1A) and constitutive function of the cell (β-actin). Changes in gene expression are considered as an integral part of the cellular response to thermal stress. It is widely accepted that HSPs are the best candidates whose expressions are affected by heat shock, moreover it has been recently shown that thermal stress also induced expression of a number of non-HSP genes such as MnSOD (30). Briefly, heat stress impacts different cell functions such as: 1) inhibition of DNA synthesis, transcription, RNA processing and translation; 2) disruption of cytoskeletal component; and 3) changes in membrane permeability that cause an increase in Ca^{2+}, Na^{+} and H^{+} (39, 40).

HSP A1A, a member of the inducible heat-shock family, can protect oocytes against different drastic conditions, including oxidative stress (41, 42). In the current study, we demonstrated that the expression of HSP A1A reduced in vitrified-warmed MII when compared to control oocytes; we detected HSP A1A only in the control and VSI groups. Boonkusol et al. also reported similar results after vitrification with straw. This might be a sign of decreasing oocyte viability following vitrification

(6, 34). Moreover, the high toxicity of cryoprotectant in the VSII group can be another reason. The results of this study showed higher expression of MnSOD in vitrified-warmed oocytes as compared to control oocytes. It is notable that heat shock can affect non-HSP genes such as MnSOD and increase expression of this gene in rat cardiac myocytes which is due to an effect on the redox state and low oxidative capacity of this tissue (43, 44). Moutassim et al. demonstrated that the freeze-thaw process can be a main source of producing free radical oxygen (FOR) in oocytes and embryos (29).

Besides, FOR can affect expression of many genes, including antioxidative enzymes causing it to up or down regulate (45). Oxidative stress may impair in cellular function and affect further developmental competence of oocytes (46). Oxidative stress can cause DNA instability in mouse oocytes. Moreover, Bilodeau et al. have reported that during the cryopreservation the activity of SOD was reduced to 50% in bovine spermatozoa (47). Therefore high expression of MnSOD in vitrified-warmed oocytes can be a defense mechanism against oxidative stress (46). All vitrification processes may be induced by stress. There are several critical factors that affect vitrification such as the concentration and toxicity of cryoprotectants, protocol and cryodevice. Therefore it is important to choose a proper approach in order to minimize oxidative, osmotic and heat stress (48). For the first time, Kuwayama et al. designed the cryotop and reported a 91% survival rate, 81% cleavage rate and 50% blastocyste rate result to 11 live births in humans (14). According to further studies that have been recently completed, the efficiency of the cryotop technique for cryopreservation of sensitive samples, such as human oocytes was confirmed. Additionally, high survival and blastocyte production rates were achieved (20, 49).

In the current study, an attempt was made to increase the cooling rate by using the minimum volume cooling method (cryotop). Additionally we attempted to decrease cytotoxicity of the cryoprotectant with the vitrification solution and therefore reduce stress. For the first time, Ishimori et al. successfully used a combination of EG and DMSO. In order to prevent intracellular ice crystal formation, permeating cryoprotectants are used. On the other hand, using a combination of two or three CAPs are effective to reduce the concentration and individual specific toxicity of each CAP (20).

Besides, osmotic stress during the vitrification process may alter DNA integrity and affect the expression of specific genes (3). The result that was obtained in the present study confirmed the efficiency of the cryotop method in vitrification of MII oocytes. It has been recently reported that using a mixture of 15% EG and 15% DMSO is beneficial for vitrifying human oocytes. Chian et al. also demonstrated the efficiency of a mixture of 15% EG and 15% PROH (4). During the Vvitrification procedure the equilibration time, thawing and concentration of cryoprotectant vary in relation to both the species and laboratory condition. Therefore, in the current study we compared two mixtures of 14.5% EG + 14.5% PROH and 10% EG + 10% DMSO in mouse MII oocytes. In contrast to the studies that have been undertaken by Chian et al. we found that the survival rate and developmental competence of mouse MII oocytes improved after vitrification in the mixture of 10% EG + 10% DMSO when compared to 14.5% EG + 14.5% PROH. These results support the hypothesis that the concentration of each cryoprotectant was reduced when mixed and thus can be effective in decreasing stress (50).

Conclusion

In conclusion, our finding in the present study showed the potential of the cryotop in vitrification of mouse MII oocytes. A possible reason for low developmental competence and cleavage may be the low abundance of mRNA after vitrification. In any case, it is essential to perform further studies to focus on expression of genes that are involved in the crucial practice of cells in different stages.

Acknowledgments

This project was financially supported by the Research Deputy of Tehran University of Medical Sciences and Health Services Grant No. 88-01-30-8402. We would like to thank them for their assistance. There is no conflict of interest in this study.

References

1. Cobo A, Domingo J, Perez S, Crespo J, Remohi J, Pellicer A. Vitrification: an effective new approach to oocyte banking and preserving fertility in cancer patients. Clin Transl Oncol. 2008; 10(5): 268-273.
2. Dhali A, Anchamparuthy VM, Butler SP, Pearson RE, Mullarky IK, Gwazdauskas FC. Gene expression and development of mouse zygotes following droplet vitrification. Theriogenology. 2007; 68(9): 1292-1298.
3. Huang JY, Chen HY, Park JY, Tan SL, Chian RC. Comparison of spindle and chromosome configuration in in vitro- and in vivo-matured mouse oocytes after vitrification. Fertil Steril. 2008; 90 (Suppl 4): 1424-1432.
4. Chian RC, Kuwayama M, Tan L, Tan J, Kato O, Nagai T. High survival rate of bovine oocytes matured in vitro following vitrification. J Reprod Dev. 2004; 50(6): 685-696.
5. Succu S, Bebbere D, Bogliolo L, Ariu F, Fois S, Leoni

GG, et al. Vitrification of in vitro matured ovine oocytes affects in vitro pre-implantation development and mRNA abundance Sara Succu and Daniela Bebbere contributed equally to this work. 2008, Wiley Subscription Services, Inc., A Wiley Company Hoboken.
6. Boonkusol D, Gal AB, Bodo S, Gorhony B, Kitiyanant Y, Dinnyes A. Gene expression profiles and in vitro development following vitrification of pronuclear and 8-cell stage mouse embryos. Mol Reprod Dev. 2006; 73(6): 700-708.
7. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at -196 degrees C by vitrification. Nature. 1985; 313(6003): 573-575.
8. Vajta G, Holm P, Kuwayama M, Booth PJ, Jacobsen H, Greve T, et al. Open Pulled Straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ova and embryos. Mol Reprod Dev, 1998. 51(1): p. 53-58.
9. Martino A, Songsasen N, Leibo SP. Development into blastocysts of bovine oocytes cryopreserved by ultrarapid cooling. Biol Reprod. 1996; 54(5): 1059-1069.
10. Lane M, Bavister BD, Lyons EA, Forest KT. Containerless vitrification of mammalian oocytes and embryos. Nat Biotechnol. 1999; 17(12): 1234-1236.
11. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online. 2005; 11(3): 300-308.
12. Gook DA, Edgar DH. Human oocyte cryopreservation. Hum Reprod Update. 2007; 13(6): 591-605.
13. Kuwayama M, Vajta G, Ieda S, Kato O. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. Reprod BioMed Online. 2005; 11(5): 608-614.
14. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod BioMed Online. 2005; 11(3): 300-308.
15. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology. 2007; 67(1): 73-80.
16. Lucena E, Bernal DP, Lucena C, Rojas A, Moran A, Lucena A. Successful ongoing pregnancies after vitrification of oocytes. Fertil Steril. 2006; 85(1): 108-111.
17. Kuwayama M. Oocyte cryopreservation. JMOR. 2007; (1)24: 2-7.
18. Morato R, Izquierdo D, Paramio MT, Mogas T. Cryotops versus open-pulled straws (OPS) as carriers for the cryopreservation of bovine oocytes: effects on spindle and chromosome configuration and embryo development. Cryobiology. 2008; 57(2): 137-141.
19. Vincent C, Pickering SJ, Johnson MH, Quick SJ. Dimethylsulphoxide affects the organisation of microfilaments in the mouse oocyte. Mol Reprod Dev. 1990; 26(3): 227-235.
20. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod Biomed Online. 2006; 12(6): 779-796.
21. Dhali A, Anchamparuthy VM, Butler SP, Pearson RE, Mullarky IK, Gwazdauskas FC. Effect of droplet vitrification on development competence, actin cytoskeletal integrity and gene expression in in vitro cultured mouse embryos. Theriogenology. 2009; 71(9): 1408-1416.
22. Ledda S, Bogliolo L, Succu S, Ariu F, Bebbere D, Leoni GG, et al. Oocyte cryopreservation: oocyte assessment and strategies for improving survival. Reprod Fertil Dev. 2007; 19(1): 13-33.
23. Lee SH, Kim M, Yoon BW, Kim YJ, Ma SJ, Roh JK, et al. Targeted hsp70.1 disruption increases infarction volume after focal cerebral ischemia in mice. Stroke. 2001; 32(12): 2905-2912.
24. Van Molle W, Wielockx B, Mahieu T, Takada M, Taniguchi T, Sekikawa K, et al. HSP70 protects against TNF-induced lethal inflammatory shock. Immunity. 2002; 16(5): 685-695.
25. Kwon SB, Young C, Kim DS, Choi HO, Kim KH, Chung JH, et al. Impaired repair ability of hsp70.1 KO mouse after UVB irradiation. J Dermatol Sci. 2002; 28(2): 144-151.
26. Hut HM, Kampinga HH, Sibon OC. Hsp70 protects mitotic cells against heat-induced centrosome damage and division abnormalities. Mol Biol Cell. 2005; 16(8): 3776-3785.
27. Li J, Foote RH, Simkin M. Development of rabbit zygotes cultured in protein-free medium with catalase, taurine, or superoxide dismutase. Biol Reprod. 1993; 49(1): 33-37.
28. Nonogaki T, Noda Y, Narimoto K, Umaoka Y, Mori T. Effects of superoxide dismutase on mouse in vitro fertilization and embryo culture system. J Assist Reprod Genet. 1992; 9(3): 274-280.
29. Guerin P, El Mouatassim S, Menezo Y. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum Reprod Update. 2001; 7(2): 175-189.
30. Sonna LA, Fujita J, Gaffin SL, Lilly CM. Invited review: Effects of heat and cold stress on mammalian gene expression. J Appl Physiol. 2002; 92(4): 1725-1742.
31. Succu S, Leoni GG, Bebbere D, Berlinguer F, Mossa F, Bogliolo L, et al. Vitrification devices affect structural and molecular status of in vitro matured ovine oocytes. Mol Reprod Dev. 2007; 74(10): 1337-1344.
32. Gardner DK, Lane M. Ex vivo early embryo development and effects on gene expression and imprinting. Reprod Fertil Dev. 2005; 17(3): 361-370.
33. Katz-Jaffe MG, Linck DW, Schoolcraft WB, Gardner DK. A proteomic analysis of mammalian preimplantation embryonic development. Reproduction. 2005; 130(6): 899-905.
34. Chen SU, Lien YR, Chao KH, Ho NH, Yang YS, Lee TY. Effects of cryopreservation on meiotic spindles of oocytes and its dynamics after thawing: clinical implications in oocyte freezing--a review article. Mol Cell Endocrinol. 2003; 202(1-2): 101-107.
35. Winston NJ, McGuinness O, Johnson MH, Maro B. The exit of mouse oocytes from meiotic M-phase requires an intact spindle during intracellular calcium release. J Cell Sci. 1995; 108(Pt 1): 143-151.
36. Dieleman SJ, Hendriksen PJ, Viuff D, Thomsen PD, Hyttel P, Knijn HM, et al. Effects of in vivo prematuration and in vivo final maturation on developmental capacity and quality of pre-implantation embryos. Theriogenology. 2002; 57(1): 5-20.
37. Leoni GG, Bebbere D, Succu S, Berlinguer F, Mossa F, Galioto M, et al. Relations between relative mRNA abundance and developmental competence of ovine oocytes. Mol Reprod Dev. 2007; 74(2): 249-257.
38. Fair T, Murphy M, Rizos D, Moss C, Martin F, Boland MP, et al. Analysis of differential maternal mRNA expression in developmentally competent and incompetent bovine two-cell embryos. Mol Reprod Dev. 2004;

67(2): 136-144.
39. Kuhl NM, Rensing L. Heat shock effects on cell cycle progression. Cell Mol Life Sci. 2000; 57(3): 450-463.
40. Lindquist S. The heat-shock response. Annu Rev Biochem. 1986; 55: 1151-1191.
41. Christians E, Campion E, Thompson EM, Renard JP. Expression of the HSP 70.1 gene, a landmark of early zygotic activity in the mouse embryo, is restricted to the first burst of transcription. Development. 1995; 121(1): 113-122.
42. Dong Z, Wolfer DP, Lipp HP, Bueler H. Hsp70 gene transfer by adeno-associated virus inhibits MPTP-induced nigrostriatal degeneration in the mouse model of Parkinson disease. Mol Ther. 2005; 11(1): 80-88.
43. Yamashita N, Hoshida S, Nishida M, Igarashi J, Taniguchi N, Tada M, et al. Heat shock-induced manganese superoxide dismutase enhances the tolerance of cardiac myocytes to hypoxia-reoxygenation injury. J Mol Cell Cardiol. 1997; 29(7): 1805-1813.
44. Yamashita N, Hoshida S, Taniguchi N, Kuzuya T, Hori M. Whole-body hyperthermia provides biphasic cardioprotection against ischemia/reperfusion injury in the rat. Circulation. 1998; 98(14): 1414-1421.
45. Maitre B, Jornot L, Junod AF. Effects of inhibition of catalase and superoxide dismutase activity on antioxidant enzyme mRNA levels. Am J Physiol. 1993; 265(6 Pt 1): L636-L643.
46. El Mouatassim S, Guerin P, Menezo Y. Expression of genes encoding antioxidant enzymes in human and mouse oocytes during the final stages of maturation. Mol Hum Reprod. 1999; 5(8): 720-725.
47. Bilodeau JF, Chatterjee S, Sirard MA, Gagnon C. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Mol Reprod Dev. 2000; 55(3): 282-288.
48. Succu S, Bebbere D, BogliolL, Ariu F, Fois S, Leoni GG, et al. Vitrification of in vitro matured ovine oocytes affects in vitro pre-implantation development and mRNA abundance. Mol Reprod Dev. 2008; 75(3): 538-546.
49. Katayama KP, Stehlik J, Kuwayama M, Kato O, Stehlik E. High survival rate of vitrified human oocytes results in clinical pregnancy. Fertil Steril. 2003; 80(1): 223-224.
50. Dhali A, Manik RS, Das SK, Singla SK, Palta P. Vitrification of buffalo (Bubalus bubalis) oocytes. Theriogenology. 2000; 53(6): 1295-1303.